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Natural Leukocyte Interferon-α Therapy in Patients With Chronic Granulocytic Leukemia Who Have Antibody-Mediated Resistance to Treatment With Recombinant Interferon-α

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Two patients with chronic-phase chronic granulocytic leukemia initially responded to recombinant interferon α-2a (rIFN-α-2a) but relapsed as a result of development of high-titer neutralizing antibodies to rIFN-α-2a. Both patients were subsequently treated with natural leukocyte IFN-α (IFN-α-n3), and one of the two patients achieved a durable second hematologic and cytogenetic remission. IFN-α-n3 may be considered for patients in whom antibody-mediated resistance to rIFN-α-2a develops. © 1996 Wiley-Liss, Inc.

Key words: interferon antibodies, interferon therapy, CGL

INTRODUCTION

Currently, the only curative therapy for chronic granulocytic leukemia (CGL) is allogeneic bone marrow transplantation [1]. The "cure" rate with this procedure is influenced by multiple factors, including age, graft origin and processing, timing of transplantation, and degree of human leukocyte antigen compatibility. Most recently, treatment with recombinant interferon-α (rIFN-α) has been shown to provide long-term hematologic and cytogenetic remissions in a subset of patients with chronic-phase (CP) CGL [2,3]. In addition, in the most recent study comparing treatment with hydroxyurea or IFN-α, a survival advantage was demonstrated in the patients treated with IFN-α, irrespective of cytogenetic response [3]. Therefore, IFN-α may be considered as initial therapy for patients with CGL who are not transplant candidates and for those in whom the risk:benefit ratio favors IFN-α treatment over bone marrow transplantation.

The benefit of IFN-α therapy is most pronounced in patients achieving a complete or a major karyotypic remission [2,3]. In these patients, the 5-year survival rate may exceed 80%. Unfortunately, most patients with CGL who are treated with IFN-α have an inadequate response, and some patients relapse after successful treatment. Although the mechanisms of nonresponse and relapse have not been fully elucidated, they may involve the development of neutralizing antibodies, to IFN-α in some cases [4]. In this report, we show that the benefit of IFN-α therapy may be salvaged with IFN-α-n3 in some patients in whom antibody-mediated resistance to rIFN-α develops.

METHODS

Both enzyme immunoassay and bioassay were used to detect and quantify IFN antibodies at the Schering-Plough Research Institute (Kenilworth, NJ). The enzyme immunoassay is a solid-phase assay using reagents obtained from ANAWA Laboratories (Wangen, Switzerland). In the first step, beads coated with IFN-α are incubated for 24 hr with the serum samples to capture antibodies. IFN-α peroxidase conjugate is then added, and incubation is continued for an additional 24 hr. The IFN-α conjugate binds to any IFN antibody captured during the first incubation. Subsequently, enzymatic activity is determined by incubation with enzyme substrate.

The bioassay for IFN-neutralizing activity measures the neutralization for the antiviral activity of IFN-α in an

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EMC virus/FS-4 system. The serum samples are treated at 56°C for 30 min, serially diluted, and incubated for 1 hr with 10 laboratory U/ml of IFN-α. The serum-IFN mixtures are then added to preformed monolayers of FS-4 cells and incubated overnight at 37°C. The cells are then washed and infected with EMC virus for an additional 18–24 hr. The cells are then fixed and stained and the viral cytopathic effect measured with a microtiter plate reader. The titer is the reciprocal of the serum dilution, which reduces the IFN protection to 50% cytopathic effect.

RESULTS

Case 1

CP-CGL was diagnosed in a 45-year-old man, when he presented with tuberculous peritonitis, which subsequently resolved with antituberculous treatment. The CGL-related clinical findings at diagnosis included no palpable splenomegaly, a mild normocytic anemia, thrombocytosis (platelet count, 1,091,000/μl), and leukocytosis (leukocyte count, 75,000/μl). The peripheral smear findings were consistent with CP-CGL, with no blasts and 10% myelocytes. The bone marrow examination was also consistent with less than 5% blasts, and cytogenetic studies revealed the presence of the Philadelphia chromosome (Ph) in all 20 metaphases. Therapy was treated with daily administration of rIFN α-2a (5–10 million units given subcutaneously). After 6 months of treatment, the patient achieved a complete hematologic remission and a partial cytogenetic remission with 45% suppression of Ph. The patient did not complain of any side effects from the IFN.

Unfortunately, after 11 months of rIFN-α-2a therapy, the disease relapsed with leukocytosis (leukocyte count, 29,000/μl) and reappearance of the Ph in 19 of 20 metaphases. A high titer (160) of neutralizing antibodies to rIFN-α-2a was detected with an in vitro cross-reaction to IFN-α-2b but not to natural leukocyte IFN-α. Therapy was therefore changed to daily administration of IFN-α-n3 (Alferon, human-derived natural leukocyte IFN-α; 5–10 million units subcutaneously). After 6 months of treatment with IFN-α-n3, the patient again achieved a complete hematologic remission and a minor cytogenetic response with 16% suppression of Ph. After 1 year of therapy with IFN-α-n3, the cytogenetic remission improved to 21% suppression of Ph.

Case 2

CP-CGL was diagnosed in a 68-year-old man, when he presented with atypical chest pain. At presentation, he had normal hemoglobin and platelet values, leukocytosis (leukocyte count, 23,000/μl), no palpable splenomegaly, and a peripheral blood smear consistent with CP-CGL, with no blasts and 8% myelocytes. The bone marrow was also consistent with CP-CGL, with less than 5% blasts, and cytogenetic studies revealed the presence of Ph in all metaphases.

Therapy with rIFN-α-2a (5–10 million units) was started, and the patient achieved a complete hematologic remission after 3 months. A bone marrow examination performed after 8 months of treatment showed a substantial decrease in cellularity, and cytogenetic studies showed a partial cytogenetic remission with 40% suppression of Ph. Side effects were limited to mild fatigue and occasional light headedness. Unfortunately, after 13 months of treatment, the patient relapsed with leukocytosis (leukocyte count, 40,000/μL), thrombocytosis (platelet count, 513,000/μL), and reappearance of Ph in all metaphases. At the same time, all the side effects of IFN therapy had disappeared.

A high titer (640) of neutralizing anti-IFN-α-2a antibodies had developed that cross-reacted in vitro with IFN-α-2b, but not with natural IFN-α. IFN-α-n3 treatment was substituted for rIFN-α-2a at similar doses. After 4 months of treatment with IFN-α-n3, severe fatigue developed, and treatment was discontinued; there was prompt resolution of the side effect. During treatment with IFN-α-n3, the leukocyte count remained stable and the platelet count decreased (206,000/μL), After IFN-α-n3 therapy was discontinued, both the leukocyte count (205,000/μl), and the platelet count (1,073,000/μL) increased significantly. Treatment with hydroxyurea was subsequently initiated, and the patient currently remains in CP-CGL.

DISCUSSION

Anti-IFN antibodies may develop during treatment with either recombinant or natural IFN products [5]. These antibodies are classifiable as neutralizing or non-neutralizing, depending on their in vitro capability to neutralize the antiviral effects of IFN. In the United States, there are currently two commercially available rIFN-α preparations (rIFN-α-2a and rIFN-α-2b) and a human-derived natural leukocyte IFN-α preparation (IFN-α-n3, Alferon). The two rIFNs differ from each other and the amino acid sequence of the natural product by only one amino acid.

Despite this apparently minor difference, there appears to be variable immunogenicity. In a large, homogeneous group of patients with hepatitis who were treated with comparable doses of different IFN preparations, the incidence of detectable neutralizing antibodies was about 20% for rIFN-α-2a, 7% for rIFN-α-2b, and 1% for a lymphoblastoid IFN-α preparation [5]. Two additional aspects of the study revealed that the antibody titers in patients treated with rIFN-α-2a were significantly higher

and that there was cross-reactivity of the sera to the two recombinant IFNs but not to the natural product. The development of neutralizing anti-IFN antibodies has been shown to have clinical relevance in hairy cell leukemia [6], chronic hepatitis C virus infection [7], chronic hepatitis B infection [8], carcinoid tumors [9], and CGL [4].

As part of a multicenter treatment trial, the development of anti-IFN antibodies was monitored in 163 patients with CGL [10]. Among 83 evaluable patients, 33 received rIFN-α-2b and 50 rIFN-α-2a. The rates of low-titer and high-titer IFN antibodies were 6% and 0% for IFN-α-2b and 8% and 20% for IFN-α-2a, respectively. Regardless, clinically relevant neutralizing anti-IFN antibodies also have developed during treatment with IFN-α-2b [4].

As mentioned, sera obtained from patients treated with either preparation of the rIFNs neutralize both types of rIFN-α, but not the natural product. On the basis of this information, both patients were treated with IFN-α-n3. One patient has achieved a durable, complete hematologic and minor cytogenetic response. The other patient also appeared to experience in vitro IFN activity, suggested by the development of severe side effects to IFN and control of thrombocytosis. Unfortunately, treatment was discontinued after only 4 months because of the side effects. Whether the higher antibody titer observed in the second patient influenced the inadequate response or whether the patient would have achieved a better response with a longer treatment period remains unknown.

In a previous report, a patient with CGL in whom anti-IFN antibodies developed during treatment with IFN-α-2a was subsequently treated with natural IFN and had a transient reduction of the leukocyte count and platelet count without achievement of a hematologic remission [11]. Our experience suggests that natural leukocyte IFN may provide a durable, long-term benefit in some patients who have antibody-mediated resistance to rIFNs.

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